

REMARKS

Claims 1-2, 4-5, 7-13, 15, 37, 39, 42-45 and 47-57 are pending in the application. Claims 1 and 37 are amended to more clearly define the invention. Support for the amendments appears in the specification at, e.g., page 33, lines 12-14, page 34, line 8 – page 42, line 33 and page 39, lines 12-14. No new matter has been added.

Applicants thank the Examiner for the courtesy extended during the telephonic discussion on October 5, 2004. Applicants appreciate the opportunity to discuss the issues in the application. As suggested by the Examiner, Applicants have amended the claims 1 and 37 to correspond to the Examiner's Amendments made in related application 09/986,897 and resubmit the remarks, arguments and the Declaration of Dr. Eitan Fibach previously submitted with Applicants' April 22, 2004 Response to the Office Action mailed February 9, 2004.

Rejections under 35 U.S.C. 103(a)

Claims 1-2, 4-5, 7-13, 15, 37, 39, 42-45 and 47-57 are rejected as obvious over Moore et al., Blood Cells, 20: 468-48, 1994 ("Moore"); or De Bruyn et al., Stem Cells 13: 281-288, 1995 ("De Bruyn"), each in view of Cicuttine et al. Blood 80: 102-112 (1992) ("Cicuttine") and Percival, J. Nutrition 122: 2424-2429 (1992) ("Percival I"). Applicants traverse.

Independent claims 1 and 37 (and all other pending claims which depend therefrom), have been amended to recite that the cells transplanted are "expanded undifferentiated hematopoietic cells having a reduced intracellular copper content to a patient" and as previously amended to specify that the transition metal chelator and conditions of proliferation result in (i) prolonged active cell proliferation; (ii) prolonged expansion of clonogenic cells; and (iii) maintenance of undifferentiated cells in their undifferentiated state.

As detailed below, the combination of Moore or De Bruyn with either Cicuttine or Percival I is improper, relying on hindsight. Even if made (improperly), the suggested combinations do not (and cannot) teach or suggest the claimed methods of transplanting hematopoietic cells or methods of adoptive immunotherapy by obtaining hematopoietic cells

from a donor, providing the hematopoietic cells *ex vivo* with a transition metal chelator having an affinity for copper and proliferation conditions which result in (i) prolonged active cell proliferation; (ii) prolonged expansion of clonogenic cells; and (iii) maintenance of undifferentiated cells in their undifferentiated state; thereby expanding the cells and transplanting the cells to a patient. In addition, there are secondary considerations present here, including a solution to a long-felt but unsolved need, praise for the invention, and unexpected results.

Graham v. John Deere Co., 383 U.S. 1, 17-18 (1966).

Secondary Considerations That Must Be Considered

There has been a long-felt but unsolved need for methods that permit *ex vivo* expansion of hematopoietic cells (and particularly subpopulations of clonogenic stem and progenitor populations therein). Hematopoietic cells are currently routinely expanded from bone marrow or stored umbilical-cord blood to reconstitute the immune systems of patients with leukemia or other hematologic cancers. Traditional methods of hematopoietic cell expansion have typically not yielded sufficient quantities of hematopoietic cells to treat adult patients. The methods of the present invention solves this long-felt need by providing greater expansion of hematopoietic cell populations and specifically of cells with engraftment ability such as the stem and progenitor subpopulations (e.g. CD34⁺ or AC133 cells and subsets) which increases the effectiveness of short-term and long-term engraftment when the expanded population is transplanted into patients. *See*, Fibach Declaration ¶ 7.

Applicants' claimed methods require use of a transition metal chelator. The claimed methods achieve unexpectedly superior expansion of the hematopoietic population in total, as well as unexpectedly superior selective expansion of the stem and progenitor populations (which is critical for short-term and long-term engraftment effectiveness).

As the specification demonstrates, the present invention teaches that providing hematopoietic cells with a transition metal chelator in combination with early acting cytokines results in not only an inhibition of differentiation and increased cell proliferation but greatly increases the expansion of a subpopulation of clonogenic cells (representative of the stem and

progenitor populations) and maintains undifferentiated cells in their undifferentiated state (*See*, specification at Example 1, page 34, line 8 – page 42, line 33, Table 1 and Figures 1-4). *See*, Fibach Declaration ¶ 8.

Specifically, the addition of a transition metal chelator, such as TEPA, to cultures containing an early acting cytokine, such as IL-3, surprisingly resulted in a two-fold increase in total cells, four-fold increase in percentage of CD34⁺ cells present in the total cell population and, most strikingly, a five-fold increase in clonogenic cell expansion (*See*, specification Table 1 at page 36, comparing rows 4 and 5). These results demonstrate that providing hematopoietic cells with a transition metal chelator led to excellent expansion of hematopoietic cells. Moreover, these results show the superior and preferential expansion of progenitor subsets such as clonogenic cells and CD34⁺ cells. The fold expansion of progenitor cell subsets is higher than the fold expansion of total nuclear cells. Thus, progenitor cell frequency increased among the entire cell population. The enrichment and expansion of this subpopulation of hematopoietic cells, i.e., stem or progenitor cells, is critical to improving short-term and long-term engraftment in hematopoietic cell transplantation and adoptive immunotherapy. *See*, Fibach Declaration ¶ 9.

Moreover, the results in Example 1 also surprisingly demonstrate that a transition metal chelator with affinity for copper (e.g., TEPA) greatly improved clonability in long term cultures, and in fact, the clonability of long term cultures surpass that of cells in short term cultures (*See*, specification at Example 1, page 37, lines 4-7 and Figures 3-4). This data displays the superior properties of the claimed methods, since it is essential that self-renewal be maximally prolonged in order to achieve maximal *ex vivo* expansion of hematopoietic cells. *See*, Fibach Declaration ¶ 9.

Recent studies, phase I human clinical trials, using the claimed methods have confirmed the results disclosed in Example 1 of the present invention. The trial comprised four human patients (3 Males, 1 Female) with varying diagnoses and ranging in age from 10-24 and in weight from 50-77 kg. In the trial, hematopoietic cells from donors were treated with a transitional metal chelator as described in the present invention. These results confirm the surprising and unexpected results disclosed in Example 1. Specifically, the trials showed the superior expansion of not only Mononuclear Cells (MNC) (expansion from 74 to 420.5 fold) but also the

striking and superior selective expansion of CD34⁺ cells (expansion from 1.9 to 57.8 fold). More specifically, patient 1 showed a very good 2 fold expansion, patients 3 and 4 an excellent 20.9 and 29.6 fold expansionl, respectively and patient 2 a dramatic 57.8 fold expansion of clonogenic cells.

In sum, these results demonstrate that the claimed methods result in the superior expansion of clonogenic cells, and that this in turn will result in greatly improved short-term and long-term engraftment effectiveness. *See*, Fibach Declaration ¶ 10.

Finally, Applicants have received praise for the claimed methods. As Dr. Fibach states, a scientific Abstract reporting results obtained with the claimed methods received the “Best Abstracts Award” from the American Society for Blood and Marrow Transplantation. Moreover, the improved long-term engraftment effectiveness of hematopoietic cells expanded by the process described in the instant invention has been praised in The Wall Street Journal health section. *See*, Fibach Declaration ¶ 11.

The combination of either of the primary references, Moore or De Bruyn, with either of Cicuttine or Percival I could not lead the ordinarily skilled artisan to the solution to the long-felt need (methods that permit *ex vivo* expansion of hematopoietic cells, particularly subpopulations of clonogenic stem and progenitor populations therein and the resulting improved short-term and long-term engraftment of these cells), nor to the unexpected and superior advantages (prolonged active cell proliferation, prolonged expansion of clonogenic cells and maintenance of undifferentiated cells in their undifferentiated state) that the claimed invention provides.

The Combinations Are Improper And In Any Event Are Defective

The combination of either of the primary references with either of the secondary references is improper – the ordinarily skilled artisan is not directed to the suggested combinations (for reasons detailed below) and the combinations can only be made with the impermissible application of hindsight.

Determination of obviousness cannot be based on the hindsight combination of components selectively culled from the prior art to fit the parameters of the patented invention.

Crown Operations Int'l, LTD v. Solutia Inc., 289 F.3d 1367 (Fed. Cir. 2002). In making the assessment of differences, 35 U.S.C. § 103 specifically requires consideration of the claimed invention "as a whole", it being well recognized that inventions typically are new combinations of existing principles or features. Env'tl. Designs, Ltd. v. Union Oil Co., 713 F.2d 693 (Fed. Cir. 1983). The "as a whole" instruction in 35 U.S.C. § 103 prevents evaluation of the invention part by part, such that the obviousness assessment improperly breaks an invention into its component parts (A + B + C), then uses a prior art reference containing A, another containing B, and another containing C, and on that basis alone declare the invention obvious. Ruiz v. A.B. Chance Co., 357 F.3d 1270 (Fed. Cir. 2004).

The Examiner concedes that neither Moore nor De Bruyn explicitly teach the claimed methods of hematopoietic cell transplantation or a method of adaptive immunotherapy, with the express requirements that the hematopoietic cells are provided *ex vivo* with a transition metal chelator having an affinity for copper such that the proliferation conditions and presence of chelator result in (i) prolonged active cell proliferation; (ii) prolonged expansion of clonogenic cells; and (iii) maintenance of undifferentiated cells in their undifferentiated state.

The Examiner has then applied two secondary references, Cicuttine or Percival I, to supply the claimed requirement for a transition metal chelator with affinity for copper. The Examiner has already conceded that the primary references Moore and De Bruyn do not teach or suggest the use of a transition metal chelator, and certainly not the specifically recited transition metal chelator with an affinity for copper (*See*, Office Action at page 4, fourth paragraph).

There is nothing in Cicuttine that would motivate the ordinary skilled artisan to combine its teachings with either of the primary references Moore or De Bruyn to arrive at the claimed invention. Cicuttine does not cure the deficiencies of Moore or De Bruyn; as Cicuttine does not teach or suggest culturing hematopoietic cells with a transition metal chelator with an affinity for copper and conditions that result in (i) prolonged active cell proliferation; (ii) prolonged expansion of clonogenic cells; and (iii) maintenance of undifferentiated cells in their undifferentiated state, as specifically required by the amended claims.

The ordinarily skilled artisan would not combine Cicuttine with either of the primary references for a variety of reasons.

Cicutline refers to the control of proliferation of stromal cell lines that are in turn co-cultured with certain hematopoietic cells – Zinc was added to the culture medium only to switch on the T oncogene under the control of a Zn-responsive element of a metallothionein promoter relating to the proliferation of the underlying stromal cell line (See, Cicutline at page 103, column 1, lines 25-28). Moreover, the specified culture conditions exclude the possibility that the hematopoietic and stroma cell co-cultures are initiated in the presence of zinc as the zinc is specifically washed out prior to the introduction of hematopoietic cells to the co-culture (See, Cicutline at page 104, column 2, lines 7-12). See, Fibach Declaration ¶ 13.

Cicutline does not refer in any way to use of a transition metal chelator with an affinity for copper in the culture of hematopoietic cells for (i) prolonged active cell proliferation; (ii) prolonged expansion of clonogenic cells; and (iii) maintenance of undifferentiated cells in their undifferentiated state. The Examiner states that “zinc has an affinity to copper and thus would reduce copper utilization of culturing hematopoietic cells”. The Examiner concludes that “it would have been obvious to one of ordinary skill in the art ... that culturing the cell in the medium containing zinc would reduce a capacity of hematopoietic cells in utilizing copper”. See, Office Action at page 4, fifth paragraph. The Examiner is apparently taking the position that Zinc is a transition metal chelator with an affinity for copper. As Dr. Fibach states in his Declaration, zinc is a transition metal and not a transition metal chelator with an affinity for copper, as required by the claimed methods. Moreover, as stated *supra*, the hematopoietic cells are not cultured in a medium containing zinc as suggested by the Examiner. See, Fibach Declaration ¶ 13.

Further, Applicants have carefully reviewed the discussion section of Cicutline and do not agree with the Examiner that there is support for the suggestion that zinc has an affinity for copper; in fact, the term copper is not disclosed in Cicutline. As Dr. Fibach concludes, since both zinc and copper ions are positively charged species in solution, “affinity” is not likely. See, Fibach Declaration ¶ 13.

The use of the isolated statements in Cicutline in combination with the primary references is impermissible hindsight -- Cicutline’s use of Zinc to control proliferation of a metal sensitive promoter driving oncogenic proliferation of a stromal cell line (and not the hematopoietic cells

directly or indirectly) cannot provide the requisite teaching to support combination with the primary references here.

In addition, even if the combination were proper (it is not) the combination would not lead to the claimed invention – that is because (a) there is not one word in the primary references about use of a transition metal chelator, and (b) nor is there a single word in Cicutine regarding the use of a transition metal chelator, as expressly required by the claims. Nor is there any teaching or suggestion that use of a transition metal chelator would provide the surprising and unexpected results detailed above and in Dr. Fibach’s declaration. The combination must fail.

For these reasons, the ordinarily skilled artisan would not and could not combine Moore or De Bruyn with Cicutine to reach the claimed invention.

In addition, the ordinary skilled artisan would not combine either Moore or De Bruyn with Percival I to reach the claimed invention. Even if the combination were proper (it is not), Percival I does not cure the deficiencies of Moore or De Bruyn.

There are many deficiencies in Percival I that the ordinarily skilled artisan would recognize, detailed below, which would teach away from combination with the primary references.

First, the Examiner has noted, “applicant and the examiner differ on interpretation” of the claim language and certain language in Percival I. Specifically, Percival I states:

“In summary, incubating HL-60 cells with TEPA resulted in copper-deficient cells without loss of viability or alteration in the stage of differentiation.” (See, Percival I at page 2428, column 2, lines 34-36)

The Examiner concludes that this statement supports the Examiner’s conclusion that Percival I teaches “culturing conditions using defined growth medium conditions that will stimulate growth while inhibiting differentiation” (See, Office Action at page 5, first paragraph, lines 1-2) and further states that “chelating copper with TEPA will inhibit differentiation” (See, Office Action at page 5, first paragraph, lines 5-6).

Applicants disagree with this characterization of Percival I. This statement in Percival I is correctly interpreted to mean that TEPA had no effect on the differentiation of the single cell line – HL-60 cells – used in Percival I. Other statements in Percival I clearly support this interpretation. The authors of Percival I hypothesized that TEPA may be an inducer of differentiation, and specifically test TEPA to determine if TEPA acts as an inducer of HL-60 differentiation:

“It was necessary ... to determine whether incubating the HL-60 cells with the copper-chelating compound would result in differentiation ... [i]ncubating cells with TEPA did not affect the respiratory burst activity, demonstrating that neither the compound nor the chelation of copper resulted in cell differentiation.” (See, Percival I at page 2428, column 1, lines 22-34). See, Fibach Declaration ¶ 14.

Applicants again direct the Examiner to a later publication by Percival (Am. J. Clin. Nutr. 67:1064-68, 1998)(“Percival II”). Percival II is crystal clear on the results of Percival I (noted as reference 27 in Percival II).¹

¹ Percival II is quite clear – it first demonstrates that “copper supplementation enhanced retinoic acid-induced differentiation [of HL-60 cells]” (See, Percival II at page 1066S, column 2, lines 5-6, referring to the studies of Bae and Percival, J. Nutrition 123: 997-1002 (1992)) and then based on that finding Percival II asks the following question: “If copper is removed from the cell is differentiation impaired or prevented?” (See, Percival II at page 1066S, column 2, lines 7-8). The author of Percival II hypothesizes, similarly as the Examiner, stating “[w]e hypothesized that if copper is essential for differentiation, then chelation of copper with TEPA should prevent the cells from differentiating.” (See, Percival II at page 1066S, column 2, lines 8-11). Percival II then cites the studies of Percival I showing that this hypothesis is incorrect stating, “Cells incubated with TEPA and retinoic acid produced the same amount of superoxide anion as did the cells with retinoic acid, **indicating that differentiation had occurred.**” (Emphasis Added) (See, Percival II at page 1066S, column 2, lines 15-18). Percival II further states that “So whereas our TEPA model is useful in some studies related to manipulating copper concentrations and Cu/Zn SOD activity, **it does not prevent HL-60 cells from differentiating.**” (Emphasis Added) (See, Percival II at page 1066S, column 2, lines 28-30). This teaches away. In fact, it teaches precisely the opposite result achieved with the claimed methods. In fact, the inability of TEPA to inhibit differentiation prompted the author to develop a different model to study copper’s role in the differentiation of HL-60 cells, “The lack of effect of TEPA on HL-60 differentiation prompted us to develop a mouse model to continue our investigation of copper’s role in granulopoiesis.” (See, Percival II at page 1066S, column 2, lines 31-33). See, Fibach Declaration ¶ 15.

Moreover, Percival I teaches nothing or teaches away (nor could it since it refers only to HL-60 cells) with regard to (i) prolonged active cell proliferation; and (ii) prolonged expansion of clonogenic cells, as expressly recited in the amended claims. The differences between these express recitations in the claims and the disclosure in Percival I are detailed below:

- (i) prolonged active cell proliferation (Percival I teaches away, stating that for HL-60 cells, TEPA “did not affect the growth rate” – i.e., expansion as unaffected by a transition metal chelator);
- (ii) prolonged expansion of clonogenic cells (Percival I teaches away, not referring to this population of cells at all, and referring only to HL-60 cells, whose expansion, unlike the claim recited population, was unaffected by a transition metal chelator. HL-60 is an immortalized cell line, which proliferates continuously without undergoing terminal differentiation under normal culture conditions (such as provided in Percival I), unless supplemented with a specific differentiation inducer such as retinoic acid. Moreover, the HL-60 cell line of Percival I was derived from a patient with acute promyelocytic leukemia, cannot be isolated from a normal donor or another patient, nor can it be introduced into a human host because of its leukemic nature). See, Fibach Declaration ¶ 16.

In sum, for the above reasons, the ordinarily skilled artisan would not have combined either of the primary references with Percival I, especially since Percival I either does not teach at all, or teaches away on virtually every aspect of the expressly recited claim limitations.

Finally, the combination of either of the primary references, Moore or De Bruyn, with Percival I could not lead the ordinarily skilled artisan to the solution to the long-felt need (methods that permit *ex vivo* expansion of hematopoietic cells, particularly subpopulations of clonogenic stem and progenitor populations therein and the resulting improved short-term and long-term engraftment of these cells), nor to the unexpected and superior advantages (prolonged active cell proliferation, prolonged expansion of clonogenic cells and maintenance of

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undifferentiated cells in their undifferentiated state) that the claimed invention provides (as detailed above).

The § 103 rejections should be withdrawn.

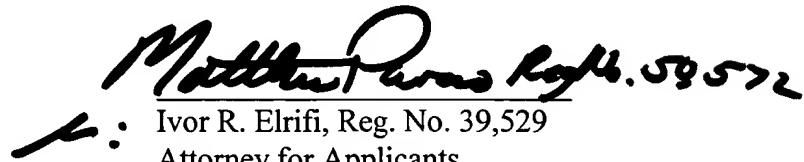
CONCLUSION

Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

This Response and Amendment accompanies a Request for Continued Examination, a Petition for a One-Month Extension of Time and the required fees under 37 C.F.R. §§ 1.17(e) and 1.17(a)(1). With this extension, this Request for Continued Examination and Response and Amendment is due on or before Monday, November 1, 2004. Applicants believe no additional fees are due. However, the Commissioner is authorized to charge any fees that may be due, or credit any overpayment of same, to Deposit Account No. 50-0311, Reference No. 24024-501 CON.

Respectfully submitted,

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